

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : A61K 31/40, 9/50	AI	(11) International Publication Number: WO 95/03043 (43) International Publication Date: 2 February 1995 (02.02.95)
(21) International Application Number: PCT/US94/08365 (22) International Filing Date: 25 July 1994 (25.07.94) (30) Priority Data: 08/097,443                      26 July 1993 (26.07.93)                      US (71) Applicant: STATE OF OREGON, acting by and through THE OREGON STATE BOARD OF HIGHER EDUCATION on behalf of THE OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098 (US). (72) Inventors: LEWY, Alfred, J.; 4717 S.W. Fairhaven Drive, Portland, OR 97221 (US); SACK, Robert, L.; 1825 S.W. Elizabeth Street, Portland, OR 97201 (US); PARROTT, Keith, A.; 3286 S.W. Long Street, Corvallis, OR 97333 (US); AYRES, James, W.; 1173 Charlemagne Street, Corvallis, OR 97330 (US). (74) Agent: NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: SUSTAINED RELEASE ORAL COMPOSITIONS CONTAINING MELATONIN		
(57) Abstract  Methods for treating circadian rhythm disorders and sleep disorders are described. The method involves oral administration of a sustained release composition of melatonin to produce a normal melatonin pattern when the normal pattern has been disrupted or is missing.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## SUSTAINED RELEASE ORAL COMPOSITIONS CONTAINING MELATONIN

## BACKGROUND OF THE INVENTION

1. Field of The Invention

5       The field of the invention disclosed in this application relates to the regulation of circadian rhythms in humans, and to the synchronization of circadian rhythms with the external environment. Specifically, this invention describes a method to achieve a chronobiologic effect in humans. In particular, this invention relates to the reestablishment of synchrony between a human's endogenous  
10       biological circadian rhythm and the external environment (including the sleep-wake cycle) after its disruption in any of a number of ways.

In addition, this invention relates to methods and compositions to achieve normal melatonin patterns in humans having melatonin deficiency syndrome.

15       2. Background of The Related Art

The phenomenon of circadian rhythms in biology is well known, and circadian rhythms are exhibited by all eukaryotic plants and animals, including man. Biological rhythms are periodic fluctuations in biologic properties over time; these include circadian as well as seasonal variations. Circadian, or  
20       approximately 24-hour, rhythms include the production of biological molecules such as hormones, the regulation of body temperature, and behaviors such as wakefulness, sleep and periods of activity. In nature, circadian rhythms are closely tied to environmental cues that impose a 24-hour pattern on many of these fluctuations. An example of longer patterns that also involve the effect of  
25       hormones includes the menstrual cycle. Experimental inquiry has established that when environmental cues are absent, most apparent 24-hour circadian rhythms have a periodicity of approximately 25 hours. Circadian rhythms that are not regulated by environmental cues are said to be free-running. The regulation of circadian rhythms by signals from the environment is said to involve entrainment  
30       of the circadian rhythms. The environmental signals that effect entrainment have been termed zeitgebers, an example of which is the light-dark cycle.

It is thought in the art that the control of circadian rhythms in mammals

is mediated by a portion of the brain called the superchiasmatic nuclei (SCN). One of the major circadian rhythms, that of wakefulness and sleep, is mediated by a feedback loop involving the retina, the SCN and the pineal gland. Melatonin, an indoleamine, N-acetyl-5-methoxytryptamine, is a neurohormone secreted primarily by the pineal gland in a circadian rhythm. This neurohormone is believed to be a physiological zeitgeber in some species.

The disruption of circadian rhythms in either timing or amplitude can reflect and possibly result in a number of pathophysiological states in humans. Disruption of normal circadian rhythms can occur when the timing of melatonin production, or the amplitude or duration of the melatonin pattern is abnormal. For example, the former is commonly associated with jet lag and the latter with aging.

As with all hormones, a disruption of a normal hormonal pattern can result in a variety of physiological changes and in many apparently unrelated outcomes. These outcomes can be improved by modifying, restoring, or mimicking the normal hormonal pattern. Although the definitive human physiological function of melatonin remains unknown, it has been suggested to have a role in, for example, sleep, appetite, reproduction, contraception, thyroid function, wool or hair production in animals, breeding, aging, depression, schizophrenia, sudden infant death or crib death syndrome, migraine headache, jet lag, seasonal affective disorder, shift worker syndrome, melatonin deficiency syndrome, pre-menstrual syndrome, mammalian breast and other carcinomas, and to have immunostimulant and immunomodulatory effects.

U.S. Patents Nos. 4,665,086 and 4,600,723 teach the use of melatonin to alleviate the symptoms of jet lag. These patents teach the use of 1-10 mg of melatonin, taken at destination bedtime, and again upon premature awakening in the middle of the night. A number of examples are disclosed in these patents, all involve travelers who take these doses of melatonin at destination bedtime and report the alleviation of the symptoms of jet lag.

It is known that melatonin in high pharmacological doses increase tiredness and the tendency to sleep in some people (see Arendt *et al.* Neurosci. Lett. 45:

317-325, 1984; Arendt *et al.* CIBA Found. Symp. 117:266-283, 1986).

Arendt *et al.*, *Ergonomics* 30: 1379-1393 (1987), disclose the administration of melatonin to alleviate jet lag. Exogenous melatonin is administered orally from 4 to 6 hours prior to the human's normal bedtime. This schedule of melatonin was reported subjectively both to improve sleep quality and decrease sleep latency and to promote a more rapid reestablishment of the circadian rhythms of endogenous melatonin production.

Armstrong *et al.*, *Experientia* 45: 932-938 (1989), disclose that in rats the effects of exogenous melatonin administration on the circadian rhythm of the sleep/wake cycle depend on the time of administration relative to the sleep/wake cycle, and that the effect was greatest when exogenous melatonin was administered a few hours before the effective start of the nocturnal activity cycle. However, these authors were unable to demonstrate phase-delay shifts; they also did not relate the timing of exogenous melatonin administration to the time of the endogenous melatonin onset.

Gwinner and Benzinger, *J. Comp. Physiol.* 126:123-129 (1978), teach that daily injections of melatonin can entrain the activity/rest cycle in birds.

Underwood, *J. Pineal Res.* 3:187-196 (1986), disclosed a phase response curve (PRC) for melatonin in the lizard *Sceloporus occidentalis*; they, as well, did not relate the timing of exogenous melatonin administration to the time of the endogenous melatonin onset.

Mallo *et al.*, *Acta Endocrinol.* 119:474-480 (1988), teach that administration of 8 mg of melatonin to humans, one hour before bedtime over a course of four days, results in a slight phase advance three days after cessation of the melatonin treatment. However, not all rhythms advanced and there was no change in the melatonin rhythm the day after the last day of exogenous melatonin administration.

The prior art is replete with examples in medical therapy involving physiological replacement of natural occurring hormones to mimic hormonal patterns. Hormonal patterns are described by the timing (including onset time, offset time, and duration time) and amplitude of concentrations in the body or

blood. Thus, hormonal treatment may include increasing the amplitude by administering exogenous hormone if the quantity produced internally is too small, increasing the duration if it is too short, or changing the onset or offset time.

Administration of hormones requires relatively uniform bioavailability since normal ranges of hormone concentrations in the body are relatively narrow and reproducible within their circadian or normal rhythm patterns. Hormones are part of complicated feedback physiological activity loops, often resulting in a variety of outcomes depending on the stability of hormonal concentrations and phasic patterns. Products that result in large variations in availability of these agents are unacceptable in medical practice. Some estrogen hormonal products, for example, have been removed from the market because of unacceptable variations in blood concentrations and bioavailability.

Melatonin production by the pineal gland is essentially non-existent during the daytime. Sometime after dusk (often about 9 P.M.), melatonin levels increase 10- to 50-fold; the levels then decrease several hours later, either because of an endogenous mechanism or because of exposure to light in the morning, whichever happens first.

Strassman *et al.*, *J. Endocrin. Met.*, 66: 847-852 (1987) showed that melatonin plasma concentrations began rising steadily during normal sleep from below 10 picograms/ ml (pg/ml) before 10 P.M. to about 40 pg/ml at 2 A.M., remained relatively sustained between about 40 and 55 pg/ml for about three hours until 5 A.M., and then declined back toward lower levels in the later morning.

Van Coevorden *et al.*, *Am. J. Physiol.*, 260 (Endocrinol. Metab. 23): E651-E661 (1991), demonstrated that melatonin concentrations in normal young subjects ranged from about 58 to 92 pg/ml during the night with an average of 70 pg/ml while in the elderly the range was about 23-46 pg/ml with an average of about 37 pg/ml (data converted from reported concentrations originally presented in pM/l). This pattern, known as the daily rhythm of melatonin, is repeated every day with the onset time and offset (shut-off) time changing very little each day depending on the time of daylight and dusk.

Lane and Moss, *J. Clin. Endocrinol. Metab.*, 61: 1214-1216 (1985), disclosed that the increase in and maintenance of melatonin blood concentrations is due to its production by the pineal gland while its rapid clearance from the blood after production stops is due to rapid metabolism by the liver.

5 Iguchi *et al.*, *J. Clin. Endocrinol. Metab.*, 54: 1025-1027 (1982), reported the half-life of melatonin to be about 45 minutes following a 30-second injection in healthy Asian subjects. However, Mallo *et al.*, *J. Clin. Pharmacol.* 38: 297-301 (1990), reported the half-life to be much shorter following bolus injections in European subjects, only about 28 minutes. The authors of the  
10 European study state that a difference between Asian and European people in their ability to metabolize melatonin cannot be ruled out as a possible explanation for these data.

Intravenous and oral administration of melatonin has usually involved pharmacological doses, so-called because they produce concentrations of  
15 melatonin that are up to several hundred times normal. After administration of a pharmacological dose, the drug is cleared from the body in a short time (not sustained so as to mimic a normal pattern).

Doses of melatonin in the range of 2 mg to 2,500 mg are considered pharmacological and not physiological. Melatonin is also remarkably non-toxic  
20 in large doses. Nevertheless, smaller doses are preferred over such pharmacological doses.

Although remarkably non-toxic, it is possible that immediate release pharmacological (large) doses of melatonin that fail to mimic normal patterns may produce down-regulation of melatonin receptors, *i.e.*, such doses may produce a  
25 hormonal feedback loop effect. For example, it has been shown in hamsters that melatonin plasma levels in the physiologic range were effective while supraphysiologic doses (25 times greater than physiologic) were not effective. Also, Lane and Moss have suggested that high plasma concentrations of melatonin (expected to be five times normal) in liver disease patients produce a negative  
30 feedback effect to decrease melatonin production.

Moreover, it is generally considered good medical practice to administer

the lowest effective dose of a drug, even a non-toxic drug, that will produce the desired effect. This is especially important if the plasma concentration patterns are shown to be highly variable such that a large dose of drug may peak very high in some subjects so that the peak concentration is outside normal physiologic concentrations. Further, economic factors weigh in favor of reduced doses. In addition to product cost, large doses may require extra doctor visits for dosage adjustment because of, for example, down-regulation of melatonin receptors.

Fifty milligram oral immediate release melatonin tablets, for example, produce average plasma melatonin concentrations of about 80,000 pg/ml, *i.e.*, about 1600 times normal. This melatonin dosage form does not produce a sustained pattern of release: the concentration drops to about 1/4 of the peak concentration in only about 3 hours. The bioavailability from oral doses containing at least 2.5 mg of melatonin is normally about 3% to 9%. However, bioavailability has been reported to be as high as 23% or 76% from an 80 mg dose. Similar decreases in bioavailability have been associated with reduced dosages of drugs such as melatonin that undergo extensive first pass metabolism. Furthermore, the fraction of the orally ingested dose absorbed was reported to vary by 25-fold among study subjects by F. Waldhauser *et al.*, in *The Pineal Gland*, Russell Reiter, editor, Raven Press, New York, 1984, p. 359. They also noted that peak concentrations varied about 40-fold.

Such variations are not generally acceptable in medical practice and present serious problems in utilizing melatonin orally, even as an immediate release product. The problem is even more severe in hormone replacement or supplementation therapy where approximately normal hormonal patterns are desired. In such cases, 25-fold variation in bioavailability prevents development of a product to shift hormonal amplitude of a phasic curve into a normal range.

Administration of an oral sustained (slow) release formulation containing 2 mg of melatonin was shown by Aldhous *et al.* *Br. J. Clin. Pharmac.*, 19: 517-521 (1985) to not be useful for mimicking normal melatonin patterns. The results were highly variable and plasma melatonin patterns were unlike normal melatonin patterns in both amplitude and duration. The maximum plasma concentrations,



for example, were about 900 pg/ml in some cases while peaks occurred at either 2, 3, 4, 5, or 7 hours following the dose. In other cases the melatonin concentration was so low as to produce essentially no melatonin pattern over 4 or even 7 hours. In two of five cases the melatonin concentrations were about 9-fold normal and for some the concentrations were less than one-fifth of normal. Aldhous *et al.*, further teach that the nutritional state of the individual greatly influences the melatonin pattern produced and that careful control of nutrition is necessary to control peak concentrations and the pattern of melatonin. Still, the best results from non-fasting subjects indicate that oral slow release melatonin cannot be used to mimic normal melatonin patterns.

Bioavailability from the 2 mg sustained release formulation is not reported. However, Aldhous' results appear to indicate that the sustained release formulation provides no more than about 15% of the bioavailability expected from the 2 mg immediate release tablet (about 3% to 6% as taught by Lane and Moss). Thus, the prior art teaches that there is less than 1% bioavailability from oral sustained release melatonin.

In certain animals, including elderly rats and some elderly humans, nighttime melatonin production is reduced from what is considered normal. This reduced melatonin production is termed Melatonin Deficiency Syndrome.

European Patent Application No. 0 518 468 A1 provides a method for correcting a melatonin deficiency syndrome comprising administering to a patient having the syndrome, over a predetermined time period including at least part of the nocturnal period, an amount of melatonin in controlled-release form, such that the melatonin is released according to a profile which, taking into account the existing profile, simulates the profile in plasma of a human being having a normal endogenous nighttime melatonin profile.

Entrainment and regulation of the melatonin circadian rhythm has thus been demonstrated in a number of animal species. The present inventors are the first to disclose a PRC for melatonin in a human. The ability to effect an actual change in phase of the circadian rhythm would be useful for the alleviation of a number of circadian rhythm related disorders, as will be further discussed in the

embodiments below. This application discloses a method to advance or delay the onset of the production of endogenous melatonin, and hence actually affect the regulation of an endogenous circadian rhythm in man.

5

### SUMMARY OF THE INVENTION

This invention relates to a method for achieving a chronobiologic effect in a human. This effect is achieved by regulation of a human's circadian rhythm. Specifically, the circadian phase-shifting effect is achieved by the administration of exogenous melatonin, which naturally occurs in the human only during the night. The timing of the nighttime onset of melatonin production in a particular individual will vary from person to person. The circadian rhythm of melatonin production in a human is entrained principally by the (bright) light-dark cycle and reflects a variety of other biological properties which vary with a circadian rhythm. The method of the invention entails the phase-shifting of the circadian rhythm by administration of exogenous melatonin. More specifically, the method of the invention involves the administration of a particular dosage of melatonin to the human. The particular dosage is lower than dosages taught by others, and is designed to achieve melatonin levels which are substantially equal to physiological levels in the human. Further, the method of the invention relates to the timing of the administration of the dosage of melatonin to the human. The timing of this dosage in the human is described to specifically phase-shift the circadian rhythm of endogenous melatonin production. The method described in the invention can be used to advance or delay the phase of the circadian rhythm of melatonin production in the human. In this way, the present invention is able to alleviate jet lag and other circadian rhythm disorders of both the phase-delay and the phase-advance types.

It has been discovered that the time of administration of exogenous melatonin relative to the time of endogenous melatonin onset is critical to the production of the appropriate phase-shifting effect. The time of administration of exogenous melatonin must be carefully set relative to the time of endogenous melatonin onset, preferably from more than 4 hours to about 8 hours before

endogenous melatonin onset when a phase advance is desired, and about 9 hours to about 17 hours before endogenous melatonin onset when a phase-delay is desired. Since the endogenous melatonin onset typically occurs about 2 hours prior to when the normal sleep phase should begin, exogenous melatonin should be preferably administered from more than 6 hours to about 10 hours prior to the commencement of the patient's normal sleep phase when a phase-advance is desired, and about 11 hours to about 19 hours prior to the commencement of the patient's normal sleep phase when a phase-delay is desired. The preferred time of administration of exogenous melatonin for a particular individual human will be unique, depending on the individual's time of endogenous melatonin onset, which can vary markedly from individual to individual (between 7 PM to 11 PM for most individuals).

This invention also provides methods to mimic a normal melatonin pattern when the melatonin pattern is diminished because of a melatonin deficiency. This aspect of the invention contemplates administration of a sustained release melatonin formulation to a patient suffering from a deficiency of melatonin where the formulation comprises (a) a sugar-based core comprising less than about 1 mg of melatonin where the melatonin is about 0.05 to 2% by weight of the core; and (b) about 5 to 25% by weight of ethylcellulose coating comprising ethylcellulose and a plasticizer.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 discloses the experimental results of part 1 of Example 3, i.e., the effect of immediate release melatonin administration on endogenous melatonin onset.

Figure 2 is a graph of time in hours post-administration of a formulation comprising sustained and immediate release melatonin versus plasma melatonin concentrations for 0.8 mg (circles), 0.4 mg (squares), and 0.2 mg (triangles) in elderly (filled symbols) and young (open symbols) individuals.

Figure 3 discloses the experimental results of Example 5, and shows the relationship between the degree of phase shift obtained and the interval time of

administration of exogenous melatonin and the endogenous dim light melatonin onset (DLMO).

Figures 4 and 5 disclose the experimental results of part 1 of Example 6.

Figures 6 and 7 disclose the results of part 2 of Example 6 and are graphs of the time of melatonin administration versus phase shift in hours.

Figure 8 is a graph of melatonin onset versus day of study for a patient administered 0.4 mg sustained release melatonin at 9 P.M.

#### 10 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

By physiological levels or patterns is meant melatonin concentrations in the blood that mimic or are similar to normal concentrations in terms of timing, amplitude and duration.

By melatonin amplitude is meant a specific concentration of melatonin in the blood.

By melatonin duration is meant the length of time that a specific concentration of melatonin is present in the blood.

By onset time is meant the time [clock time or circadian time (defined below)] that a specific concentration of melatonin is reached in the blood. By circadian time is meant the time some internal physiological event that occurs at some predictable time relative to the endogenous circadian pacemaker. For example, the onset of melatonin production occurs at circadian time (14 in most individuals).

By offset time is meant the time (clock time or circadian time) that a specific concentration of melatonin is no longer present in the blood.

By normal melatonin levels is meant ranges of melatonin such as those taught by Van Coevorden *et al.*, and Strassman *et al.*

By a deficiency of melatonin is meant a melatonin concentration below the average normal concentration present in an individual at the time sleep is desired. Such melatonin deficiencies may be present when the individual is unable to produce a normal nighttime melatonin concentration. Low melatonin

concentrations are common in the elderly and frequently have a low quality of sleep usually in combination with a decreased duration of sleep. In addition, a deficiency of melatonin at "bed time" typically exists in an individual suffering from jet lag.

5           When such low nighttime or bedtime concentrations of melatonin are present, the individual's melatonin or circadian rhythm is dispersed or shifted out of phase. Both of these circadian rhythm problems are addressable using the method of the present invention. Thus, the present invention encompasses the administration at the time sleep is desired of a sustained release melatonin  
10           composition that yields improved amounts of bioavailable melatonin and unexpectedly provides adequate melatonin levels in individuals suffering from a deficiency of melatonin.

          Administration of sustained release melatonin or immediate release melatonin formulations corrects melatonin or circadian rhythms that are dispersed  
15           or shifted out of phase. When an individual is unable to produce a normal nighttime melatonin concentration, the administration of a sustained release formulation sets the individual's phase, *i.e.*, the formulation provides a phase shift. Similarly, when a human is experiencing jet lag, administration of either sustained or immediate release melatonin at a specific, critical time effects a phase  
20           shift.

          The amount of immediate release melatonin administered to the human patient should be sufficient to achieve the desired circadian phase-shifting effect. In a preferred embodiment of this invention, a dosage of about 0.25 mg to about  
25           75 mg, preferably about 0.75 mg, and most preferably about 0.50 mg, of exogenous immediate release melatonin is used to effect the desired change in phase of the circadian rhythm of endogenous melatonin production. In a preferred embodiment, the total dose of immediate release melatonin is given in two or more smaller portions to the human patient over an interval of about two hours if the person is awake. One dose time is preferred if the person is asleep.

30           Pharmaceutical quality melatonin is commercially available. The dosage of melatonin may be administered orally, by injection, via a transdermal patch or

by implantation of a reservoir designed to release a steady dosage of melatonin over time. In a preferred embodiment of this invention, melatonin is administered orally.

5 In a preferred embodiment of this invention, a phase advance in the circadian rhythm of endogenous melatonin production is effected by the administration of an amount of exogenous melatonin sufficient to achieve the phase advance from more than 6 hours to about 10 hours, preferably from about 7 to about 10 hours, most preferably about 8 hours, before the human's normal sleep phase should begin. This is typically from about 4 hours to about 8 hours, most preferably about 6 hours, before the patient's endogenous melatonin onset.

10 A phase delay in the circadian rhythm of endogenous melatonin production is effected by the administration of an amount of exogenous melatonin sufficient to achieve the phase delay from about 11 to about 19 hours, most preferably from about 12 to about 16 hours, prior to when the human's normal sleep phase should begin. This is typically from about 9 hours to about 17 hours, most preferably from about 10 to about 14 hours, before the patient's endogenous melatonin onset.

15 The amount of melatonin administered in the sustained release formulation to the human patient suffering from a melatonin deficiency should be sufficient to produce a normal nighttime melatonin amplitude, *i.e.*, a plasma concentration approximating those in normal individuals at night. In a preferred embodiment of this invention, a dosage of about 0.025 mg to about 1 mg, more preferably from about 0.05 to 0.75 mg, and most preferably about 0.1 mg to about 0.4 mg, of sustained release melatonin is used to treat the patient and, thus, to mimic a normal melatonin pattern in the human during the time sleep is needed. Sustained release formulations containing higher amounts of melatonin will provide longer durations of release and, in addition, higher melatonin amplitudes in the patient.

25 It has unexpectedly been discovered that an oral sustained release melatonin formulation provides a sustained plasma concentration of melatonin over a period of at least three hours, and the dose required to achieve this concentration is substantially less than 1 mg. The sustained release melatonin formulation of the invention comprises (a) a core comprising 0.05 to 2% by

weight of melatonin; and (b) about 5 to 25% by weight of ethylcellulose coating comprising ethylcellulose and a plasticizer. This formulation releases melatonin over time such that a normal nighttime amplitude of melatonin in the body is mimicked. The sustained release melatonin composition surprisingly provides at least about 5% bioavailable melatonin. In more preferred embodiments, the composition provides at least about 50% bioavailable melatonin, and more preferably at least about 10% bioavailable melatonin.

In preferred embodiments, the plasticizer is an alkyl sebacate or a trialkyl citrate, where each alkyl is the same or different and represents straight or branched chain alkyl groups having 1-6 carbon atoms. A particularly preferred plasticizer is a mixture of dibutyl sebacate and triethyl citrate at a ratio of about 1:1 by weight.

Those skilled in the art will recognize a variety of dosage forms that may be prepared to obtain a desired release pattern. Compositions containing the sustained release melatonin formulation may also contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may be prepared to contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby modify the sustained release of melatonin. For example, a time delay material such as glyceryl monoesterate or glyceryl distearate may be employed.

Gelatin capsules may be prepared wherein the melatonin formulation is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is

mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil. In a preferred aspect of the invention, particulate beads of the sustained release formulation were loaded into a gelatin capsule. Administration of the sustained release formulation of the invention produces a melatonin amplitude that closely mimics the average nighttime pattern in terms of amplitude. This formulation produces less than a five-fold variation in the peak concentrations generated relative to normal; sustained patterns are over a period of about 3 to 10 hours where the average melatonin concentrations vary by less than five-fold. Preferred formulations according to the invention produce bioavailability of at least about 5%.

Mimicked (exogenously produced) normal melatonin patterns are defined as melatonin patterns in which the average melatonin concentration is between about one-fifth and five times normal and a sustained average concentration over a period of at least three hours where the plasma concentration does not fluctuate more than about five-fold. In preferred embodiments, the oral compositions produce a minimum concentration of at least one-third normal and no more than three times normal for a peak, with a sustained concentration over at least three hours where the concentrations fluctuate no more than three fold. Preferred oral doses of sustained release melatonin produce initial melatonin concentrations of from about 25 to 300 pg/ml, average melatonin concentrations of about 70 to 80 pg/ml over a three-hour period, and no more than three-fold variation in average concentrations over the three hours. Such products may be capsules or tablets or liquids so long as they provide a relatively sustained release over a time period of about 3 to 12 hours and usefully supplement or replace normal melatonin patterns. A useful dosage form can be produced using only controlled release melatonin without immediate release hormone.

Oral sustained release melatonin is useful to replace or supplement melatonin hormone in a truly physiologic pattern. This formulation can aid Alzheimer's patients and other elderly individuals.

The present invention may be used in, but is not limited to, the following situations to achieve chronobiologic effects, alleviate circadian rhythm disorders,



and/or achieve normal concentrations of melatonin when initiation of sleep is desired *via* mimicking of normal melatonin patterns: astronauts in orbit around the earth, on missions in space to the Earth's moon or to the planets or out of the known solar system, or in training for such missions; submariners, or persons  
5 confined for research, exploration or industrial purposes below the seas; miners, explorers, spelunkers, researchers or those confined below the earth; psychiatric patients; insomniacs; the comatose, or those who need to be maintained in a state of unconsciousness for medical, psychiatric or other reasons; medical residents, nurses, firemen, policemen or all those whose duties require alertness and  
10 wakefulness at evening or nighttime hours, or those deprived of sleep for various periods because of their duties or responsibilities; the infantry, or other members of the armed forces whose duties require extreme levels of alertness and wakefulness, and who may be sleep deprived in the performance of duties; the blind or sight-impaired, or all those whose ability to distinguish differences in  
15 light and dark may be permanently or temporarily impaired; residents of the far North or Antarctica, or all those who live in a climate or climates that possess abnormal amounts of light or darkness; the aged, Alzheimer's patients, the sick, or all those who require dosages of medication at appropriate times in the circadian cycle; animal breeders, for use in controlling circadian time; in  
20 pathological conditions, such as depression, schizophrenia, sudden infant death or crib death syndrome, migraine headache, seasonal affective disorder, shift worker syndrome, melatonin deficiency syndrome, pre-menstrual syndrome, and breast and other carcinomas in mammals; to achieve immunostimulant and immunomodulatory effects, wool or hair production in animals, breeding affects, aging effects, contraception, appetite affects, reproduction, thyroid function,  
25 sleep, or to overcome the effects of jet lag.

One skilled in the art will recognize that modifications may be made in the present invention without deviating from the spirit or scope of the invention. The invention is illustrated further by the following examples which are not to be  
30 construed as limiting the invention or scope of the specific procedures described herein.

## EXAMPLE 1

1. **Preparation of sustained release  
melatonin using 8-10 mesh sugar  
seeds and 20% ethylcellulose coating**

5 Melatonin was spray layered on 8-10 mesh non-pareil sugar seeds to a concentration of 3.0 mg melatonin per gram of beads using polyvinylpyrrolidone (0.2%) and hydroxypropylcellulose (0.1%) with melatonin (0.6%) in 95% alcohol, in a spray coater. After drying, this product was then spray coated with ethylcellulose (Aquacoat\*, FMC Corp.) containing 15% of solids weight of dibutyl  
10 sebacate and 15% of solids weight of triethyl citrate to a total calculated solids coating on the melatonin and sugar seeds of 20% by weight. Although some coating is lost during the spraying process, the final coat is at least about 10% by weight of the melatonin sustained release formulation and generally greater than 15% by weight, but less than 20%.

15 These beads demonstrate sustained release of melatonin in a controlled manner over about 8 hours in a United States Pharmacopia dissolution apparatus (basket method) with a time to 50% release of about 4 hours, stirring speed 50 rpm, in enzyme-free gastric fluid for the first 2 hours and then in enzyme-free intestinal fluid.

20

2. **Preparation of sustained release  
melatonin using 18-20 mesh sugar  
seeds and 20% ethylcellulose coating**

25 Sustained release melatonin beads were prepared essentially as described in part 1 of this example except that 18-20 mesh non-pareil sugar seeds were used.

These beads demonstrate sustained release of melatonin in a controlled manner in a United States Pharmacopia dissolution apparatus (basket method) with a time to 50% release of about 1 hour.

30

3. **Preparation of sustained release  
melatonin using 8-10 mesh sugar  
seeds and 5% ethylcellulose coating**

Sustained release melatonin beads were prepared essentially as described

in part 1 of this example except that the ethylcellulose coating was applied at about 5% by weight of the formulation.

These beads demonstrate sustained release of melatonin in a controlled manner in a United States Pharmacopia dissolution apparatus (basket method) with a time to 50% release of about 1 hour.

**4. Preparation of sustained release melatonin using 8-10 mesh sugar seeds and 10% ethylcellulose coating**

Sustained release melatonin beads were prepared essentially as described in part 1 of this example except that the ethylcellulose coating was applied at about 10% by weight of the formulation.

These beads demonstrate sustained release of melatonin in a controlled manner in a United States Pharmacopia dissolution apparatus (basket method) with a time to 50% release of about 2 hours.

**5. Preparation of melatonin composition containing sustained and immediate release melatonin**

Melatonin was spray layered on 8-10 mesh non-pareil sugar seeds to a concentration of 3.0 mg melatonin per gram of beads using polyvinylpyrrolidone (0.2%) and hydroxypropylcellulose (0.1%) with melatonin (0.6%) in 95% alcohol, in a spray coater.

After drying, 0.04 mg or 0.02 mg of these immediate release melatonin beads were loaded into gelatin capsules with 0.36 mg or 0.18 mg of the sustained release melatonin beads prepared in part 1 of this example to result in a formulation containing 0.40 mg or 0.20 mg total melatonin.

A 0.80 mg melatonin formulation was prepared in a similar manner to contain 0.08 mg immediate release melatonin and 0.72 mg of sustained release melatonin.

6. **Preparation of melatonin composition  
containing sustained release melatonin**

Capsules were loaded to contain either 0.80 mg, 0.40 mg or 0.20 mg of the sustained release melatonin beads prepared in part 1 of this example were loaded into a gelatin capsule to result in formulations containing 0.80 mg, 0.40 mg or 0.20 mg total melatonin respectively.

**EXAMPLE 2**

**Detection of Melatonin Levels in Human Plasma**

Prior to collection of human blood, subjects are kept in dim light for about 5 hours (usually between 6 PM and 11PM) except for the daytime studies. An intravenous line or heparin lock is inserted in a forearm vein and 5 ml of blood drawn at specified time periods such as every 30 minutes between 7PM and 11PM. The blood samples are centrifuged for 5 minutes at 1000 g and 4 degrees C, and the plasma aspirated into silanized glass or plastic tubes. Samples are assayed immediately or frozen for later analysis. To a 1 ml aliquot of such plasma was added 15-40 picograms of N-acetyl-5-methoxy( $\alpha,\alpha,\beta,\beta$ -D-4)tryptamine as a chromatographic control. An equal volume of normal saline is added and the mixture gently shaken with 10 volumes of petroleum ether. The organic phase is removed, and melatonin and the chromatographic control extracted from the aqueous phase with 10 volumes of chloroform. The aqueous phase is then discarded, and the chloroform evaporated to dryness.

The dried extract containing melatonin and the added chromatographic control is dissolved in 0.4 ml of anhydrous acetonitrile. The melatonin and the chromatographic control are then derivatized by the addition of 25 mcl of pentafluoropropionic acid anhydride and 0.5 ml of a solution of 5% trimethylamine in anhydrous benzene and reacted at 100 degrees C for 10 minutes. The reaction products are washed sequentially with 1 ml water and 1 ml 5% ammonium hydroxide. The mixture is centrifuged briefly at 13,000 g and the organic phase withdrawn and evaporated to dryness under nitrogen. The dried extract is partitioned between 0.5 ml acetonitrile and 1 ml hexane by vigorous mixing

followed by centrifugation. The hexane layer is removed and the acetonitrile evaporated to dryness under nitrogen. This partitioning step is performed two times for each sample. The dried extract is re-partitioned for storage. The derivatives are stable and can be stored at -20 degrees C for several weeks.

5       The amount of melatonin present in each sample is determined by analysis using a gas chromatograph-mass spectrometer (GC-MS). Before injection onto the GC column, the dried derivatives are dissolved in 15 µl of ethyl acetate. Approximately half this volume was applied to a 30 x 25 micron fused silica capillary column [0.15 micron film thickness with a 1 m retention gap (DB-225,  
10       J&W Scientific, Folsom CA)]. The oven is programmed from 60 degrees C to 240 degrees C (at 25.5 degrees C/min) with helium as carrier gas (10 psi head pressure) and methane used as make-up gas (ionizer, 0.6 torr). Derivatized melatonin and chromatographic standard control are found to elute from the column after 10-14 minutes. Mass spectrographic analysis of the column eluate  
15       is then performed. Mass spectra are recorded using a Finnigan 4000-GC-CI analyzer and INCOS data system. A Finnigan PPIMCI electron multiplier with 3 kV conversion was used, signal referenced to ground. The relative signals of melatonin and the added chromatographic control are detected as m/e (mass/charge) ratios of 320 and 323, respectively. The amount of melatonin  
20       present in any unknown sample can be determined by comparisons of the ratio of the intensities of these signals to a standard curve, prepared as described using known amounts of melatonin and the chromatographic control.

### EXAMPLE 3

25

#### 1.   Phase advance in blind subject       using immediate release melatonin

Without light perception, blind people often have circadian rhythms that free run with a period greater than 24 hours. We have been successful in phase  
30       shifting a free-running circadian rhythm in at least one blind subject by administering capsules of 0.5 mg immediate release doses of melatonin orally.

A blind subject whose circadian rhythms were free-running was placed on

a three-week regimen to phase shift his circadian rhythms using administration of exogenous melatonin. The subject was given 0.25 mg of melatonin orally at 1900 and 2100 hours (clock time) every day for three weeks. The effect of exogenous immediate release melatonin administration on the time of endogenous melatonin onset is shown in Figure 1. The cumulative phase advance seen in this subject is equivalent to the phase advance obtained when a much higher dose (5 mg) was used. These results confirm that exogenous melatonin administration can effect a phase advance in a human.

2. **Phase advance in blind subject using  
immediate and sustained release melatonin**

Administration of 0.4 mg melatonin (0.04 mg immediate release and 0.36 mg sustained release) in a composition prepared as described above in part 5 of Example 1 to a blind patient who was also taking atenolol (100 mg) at 5 AM resulted in a circadian rhythm phase shift. The results shown in Figure 8 demonstrate that a low dose of melatonin formulated to be partially immediate release and partially sustained release composition effects a phase advance in a human.

**EXAMPLE 4**

**Mimicking of Normal Melatonin Patterns  
With Orally Administered Melatonin**

Melatonin formulations as prepared in part 5 of Example 1 were administered during the daytime so the resulting melatonin patterns would not be confounded by normal (endogenous) melatonin production, since melatonin is only produced at night and daytime concentrations are always less than about 10 pg/ml. These capsules were administered at 9 AM either to six young (age 22 to 37) or six elderly (age 75 to 96) healthy fasting subjects, periodic blood samples were collected, and the samples were assayed using a specific binding radioimmunoassay (RIA) for melatonin as taught by Zimmerman *et al.*, *Fertil. Steril.* 54: 612-618 (1990). Average melatonin concentrations and standard deviations were determined; the results from 0.8 mg, 0.4 mg and 0.2 mg

melatonin formulations are shown in Figure 2.

The results demonstrate that administration of an oral sustained release melatonin composition to human subjects mimics normal melatonin patterns. Circadian-phase shifting of endogenous melatonin can then occur during or  
5 following the periods when the melatonin levels are substantially equal to physiological patterns of melatonin. The example further shows that an oral controlled release composition with a low physiological dose can be administered orally to humans to replace or supplement therapy of this hormone.

Figure 2 indicate that a 0.8 mg dose of melatonin combining 0.08 mg  
10 immediate release and 0.72 mg sustained release melatonin produced plasma concentrations above about 100 mg/ml for at least 12 hours in both elderly and young individuals.

Figure 2 shows that a 0.4 mg dose of melatonin formulated as only 0.04 mg immediate release and 0.36 mg sustained release produced plasma melatonin  
15 concentrations above 100 pg/ml for about 6 hours in the young subjects and for about 7 hours in the elderly subjects. Significantly, the average peak concentrations relative to the average minimum concentrations during the sustained concentration time interval are only about 2:1 for the young and <3:1 for the elderly. These profiles and plasma concentrations are quite useful and  
20 unexpected for this dose in terms of amplitude and duration. The amplitude and duration are particularly surprising since these results are at least 10 times what were expected from 1% bioavailability.

Figure 2 shows that 0.2 mg of melatonin with 20  $\mu$ g of immediate release melatonin produces truly physiological plasma concentrations of melatonin,  
25 especially in the elderly. Because some elderly patients naturally produce some melatonin at night about one-third the melatonin of young subjects, the dose can be even further reduced in that segment of the population to about 0.15 mg or even 0.1 mg or less total melatonin, if desired to supplement normal production, in a manner that will allow plasma melatonin concentrations to mimic normal in  
30 terms of amplitude and duration.

These data show at least 5% bioavailability with a very low dose (less than

1 mg) from an oral sustained release melatonin composition with relatively non-variable results and sustained plasma melatonin concentrations that mimic a normal pattern. This type of product is useful to phase-shift circadian patterns or as a melatonin replacement or supplement in a patient who does not have a normal melatonin pattern. Administration may be at or near the patients normal sleep time, *i.e.*, when the endogenous melatonin onset should begin; or administration may be several hours (4 or more) in advance of these times in order to mimic the normal melatonin pattern during the period of interest. Such administration will normally be of a very low melatonin dose yielding bioavailability of at least 10%. This level of bioavailability is adequate to produce a physiological profile of melatonin.

#### EXAMPLE 5

##### Production of Phase Advance By Melatonin Administration

##### 1. Immediate release melatonin

The effect of exogenous melatonin administration on circadian rhythm of sighted people was tested. Eight normal subjects were treated in a two-week protocol, similar to the one used in Example 4. During the first week, the subjects were given a placebo at 1700 and 1900 hours and the time, extent and amount of dim light melatonin onset (DLMO) was measured as described in Example 2. During the second week, subjects were given placebo at 1700 and 1900 hours for two days, and then immediate release melatonin was administered orally in two doses of 0.25 mg at 1700 and 1900 hours for 4 days and the subject's DLMO determined.

Seventeen trials were conducted on the eight subjects. The results of this study are shown in Figure 3. The Figure shows the relationship between the degree of phase shift obtained and the interval between the time of administration of exogenous melatonin and the endogenous DLMO. This interval is also known as the phase angle difference or phase angle. The earlier the exogenous melatonin is administered the greater is the magnitude of the phase advance; that is, there is a positive correlation between the extent of phase advance achieved by



exogenous melatonin administration and the time interval between the time of exogenous melatonin administration and the time of endogenous melatonin onset. These results confirm that exogenous melatonin administration can effect a phase advance in humans, and that the timing of exogenous melatonin administration relative to the onset of endogenous melatonin is critically important for phase shifting the onset of the circadian rhythms.

## 2. Sustained Release Melatonin

Four sighted subjects, two female and two males, all ages 47-50, traveled from the west coast of the United States to Paris, France by jet leaving the original U.S. port of departure at about 7 AM (U.S. Pacific Time) and arriving in France at about 9 AM (France Time) the next day. Each subject was orally administered a melatonin capsule containing 0.27 mg of sustained release melatonin prepared essentially as described in part 1 of Example 1 at 1 PM (Pacific Time) on the departure day on the plane. The subjects rested and dozed during the flight. The subjects were administered a second dose of the same composition at about 10 PM to midnight bedtime in France (corresponding to about 1 to 3 PM Pacific time) on the arrival night and a third dose on the second night following the arrival at about 10 PM or midnight each night depending on the subject, that was about 1 PM or 3 PM Pacific Time. This was predicted to be about 6 hours before the normal DLMO.

All subjects had previously experienced jet lag when traveling over several time zones. It is known that their melatonin patterns would be out of phase with the local time and the DLMO phase delayed in France since the subjects were going to bed at times that were equal to the middle of the day at their departure city, and their melatonin production would be delayed and very low during the first hours in bed. Further, at 3 AM in France, it would be only 6 PM Pacific Time and it is common for travelers to awaken and have difficulty returning to sleep for several hours. All subjects experienced a tiredness and readily fell asleep following the low dose oral controlled release composition of melatonin and either slept through the night or, if they awakened, readily returned to sleep.

Such low doses of melatonin with their very low input rates are not known to be soporific. Without wishing to be bound to any particular hypothesis, it is presently believed that the physiologic outcome for the subjects who traveled to and from France and took the oral sustained release melatonin is a result of circadian phase shiftings as well as mimicking a normal melatonin pattern, and that the same outcome could not be achieved without mimicking the normal pattern.

On the return to the U.S., the subjects took the same composition of oral controlled release melatonin for the first two nights following the return. In this case, the travelers left France at about 11 AM (France Time) and arrived on the west coast of the U.S. at about 9 PM Pacific time the same day. Each subject's circadian time was still on France time, however, as 9 PM Pacific time corresponded to 6 AM. Thus, a normal melatonin night pattern was not produced by the pineal gland. In this case, the melatonin was administered at about 11 PM Pacific Time, which is about 15 hours before the normal bedtime in France or about 13 hours before DLMO. Such schedules are known to disrupt sleep patterns and the effects are commonly referred to as "jet lag". However, the study subjects reported excellent sleep patterns following the melatonin administration and a substantial reduction in the symptoms associated with jet lag.

## EXAMPLE 6

### Phase-Shifting Effect of Exogenous Melatonin Administration

#### 1. Phase advance

The effect of exogenous melatonin treatment administered at earlier times relative to the endogenous melatonin pattern was tested in sighted people. Twenty-four trials were conducted in the eight normal subjects who were treated in a two-week protocol similar to the one used in Example 5. During the first week, placebo was administered and the time of dim light melatonin onset (DLMO) was determined. Subsequently in the second week, melatonin was administered at various times prior to the time of endogenous melatonin onset, and the subject's endogenous melatonin onset was determined. The results of this

study are shown in Figures 4 and 5. Figure 4 expresses the results in terms of circadian time [assuming the DLMO occurs at circadian time (CT)14], and Figure 5 expresses the same results in terms of military time [assuming that DLMO is at 2000 hours (8 PM)]. These results show that the maximum degree of phase advance in the onset of endogenous melatonin occurred after administration of exogenous melatonin at circadian time (CT) 8, or 6 hours prior to the normal time of melatonin onset in the subjects (CT 14). This corresponds to a time of about 8-10 hours before normal bedtime in these subjects. The observed phase advance declines rapidly when exogenous melatonin is administered prior to CT 8. Between CT 8 and CT 14, the decline in the degree of advance is linear and proportional to the phase angle between time of administration and time of endogenous onset. Minimal effect, if any, on the circadian rhythm of endogenous melatonin onset is seen when the time of administration of exogenous melatonin coincides with the normal time of onset of endogenous melatonin (CT 14).

15

## 2. Phase delay

Experiments were conducted to investigate the use of exogenous melatonin to effect a phase delay. These experiments used essentially the same procedure as described above in part 1 of this Example except that the time of melatonin administration was altered.

20

A total of 30 trials using 9 subjects were performed in which exogenous melatonin was administered about 11-19 hours before normal bedtime. Administration of exogenous melatonin from about 9-17 hours before the endogenous melatonin onset resulted in the greatest degree of phase delay in the onset of endogenous melatonin production. The results of these experiments are displayed as phase response curves (PRCs) and are shown in Figures 6 and 7.

25

The results of this example demonstrate that exogenous melatonin can be administered at a specific time relative to an individual's normal sleep phase or endogenous melatonin onset to achieve phase advance or a phase delay.

30

## EXAMPLE 7

**The Effect of Physiologic Melatonin  
Administration on Sleep in the Elderly**

5 An 89-year-old woman in good health and taking no medications was determined to have low endogenous melatonin production as established by her overnight production of urinary 6-hydroxy melatonin, the major metabolite of melatonin. Her average overnight (1800 to 0900) production of 6-hydroxy melatonin was  $967 \pm 248$  (SD) ng. The average overnight production for females age 80 and above is  $6000 \pm$  S.D. 3,700 ngm, respectively, Sack et al., J. Pineal Res. 3: 379-388 (1986).

10 Sleep studies were carried out by a trained sleep technologist. An initial "diagnostic" PSG was performed on the first two nights to rule out significant primary sleep pathology. For the sleep study, a standard 16 channel montage was used: four EEG (C<sub>3</sub>A<sub>2</sub>, O<sub>1</sub>A<sub>2</sub>, C<sub>4</sub>A<sub>1</sub>, O<sub>2</sub>A<sub>1</sub>), four EMG (two for chin, one each for right and left lower extremity), one nasal air flow, two ocular movements, two for respiratory muscle movement, one for oximetry, one for body position and one for EKG. Subsequent recordings omitted the airflow and leg EMG channels. Sleep recordings were done in the subjects' own homes using portable Telediagnostics\* polysomnograph, in order to minimize effects from the  
20 unfamiliar sleep laboratory environment. Sleep records were hand-scored using conventional criteria, as described by Rechtschaffen and Kales, Manual of Standardized Terminology: Techniques and Scoring System for Sleep Stages in Human Subjects, Los Angeles; UCLA Brain Information Service/Brain Research Institute, 1968. Technicians scoring sleep were blind to the treatment condition.

25 Two nights of in-home polysomnography were done at baseline and on the first two nights and last two nights of each treatment condition. Two treatment sequences were possible:

30

(1) BASELINE → MELATONIN LDSR → PLACEBO  
2 WEEKS 2 WEEKS

5

(2) BASELINE → PLACEBO → MELATONIN  
2 WEEKS LDSR 2 WEEKS

10

(Note that LDSR stands for low-dose, slow-release). The subject was assigned randomly to treatment sequence No. 2. Placebo was administered each night at bedtime for two weeks, followed by a two-week wash-out period, followed by two weeks of 0.2 mg slow-release melatonin capsules (prepared according to part 6 of Example 1) administered each night at bedtime. Ten PSG studies were done; two at baseline and two at both the start and end of each treatment sequence.

15

20

The diagnostic (baseline) sleep studied demonstrated a lack of sleep pathology. The data from the four nights of placebo treatment were averaged and compared to the average of the four nights of the active treatment. The results are shown in the following table. In summary, the average total recorded sleep period time (lights out to lights on) was about the same between the two conditions (difference = 2.1 minutes), but total sleep time with melatonin treatment was increased 37.7 minutes. Also the wake after sleep onset was decreased by 35.7 minutes. Melatonin increased the time spent in Stage 2, 3 and 4 sleep, considered the deeper stages of sleep, and decreased the time spent in Stage 1 sleep, considered light sleep. Total non-REM and total slow wave sleep were increased. There was no apparent change in total REM sleep. There was no apparent change in sleep latency nor in REM latency. Both the number of movement arousals and transient arousals from sleep were decreased with melatonin treatment. Thus, melatonin treatment improved the quality of sleep.

25

30



TABLE I (cont'd.)

	Lights Out to S1	Lights Out to S2	Lights Out to REM	Movement Arousal during NREM	Transient Arousal during NREM
Melatonin	17.8	23.8	67.7	21.4	58.2
	19.8	19.3	50.8	28.7	85.0
Placebo	16.4	21.6	94.9	47.0	118.0
	6.6	10.9	37.2	30.4	102.5
Difference (Melatonin - placebo)	1.4	21.	-7.2	-25.6	-59.8

**EXAMPLE 8****Calculation of Bioavailable Melatonin from  
Serum Melatonin Concentrations**

Input absorption rates and cumulative amounts of melatonin absorbed (bioavailability) were estimated using an interactive computer deconvolution program and pharmacokinetic parameters similar to those reported by Iguchi *et al.* In this case the program was PCDCON, version 1.0, created at the College of Pharmacy, University of Texas. Exact bioavailability was not determined in this study since an intravenous dose was not administered as a reference standard. This method estimates the bioavailability to be at least 5% from 0.2 mg, 0.4 mg and 0.8 mg formulations of the invention.

**EXAMPLE 9****The Effect of Physiologic Melatonin  
Administration on Sleep in the Elderly**

One of the most common and distressing symptoms of Alzheimer's disease (AD) is the deterioration of the sleep/wake cycle and sleep onset and duration disturbances related thereto. Accordingly, melatonin replacement therapy using the sustained release melatonin formulations disclosed herein were used to alleviate disrupted sleep patterns in AD patients.

Six subjects free from medications affecting the CNS and diagnosed with probable AD (definitive diagnosis being only possible *post mortem*) were studied in a double-blind, placebo-controlled treatment protocol. Subjects were randomly administered melatonin (0.2mg of the slow-release formulation described in Example X above) or placebo given nightly at 2000 hrs. After two weeks under this first administration protocol, subjects underwent a two-week washout period of no melatonin or placebo administration, followed by a cross-over to the alternative administration protocol for another two weeks. Subjects' rest-activity cycles were monitored by wrist actigraphy throughout the study.

Two of the six subjects demonstrated a clinically-significant increase in nocturnal sleep in response to melatonin treatment, averaging three additional hours of nocturnal sleep during the melatonin administration leg of the protocol



and an average decrease of 37 min in daytime sleep. Pre-treatment decreased nocturnal sleep (averaging less than 5 hours) and diminished day-to-night sleep differential seemed to predict positive treatment response. In contrast, treatment non-responders (n=4) averaged greater than seven hours of nocturnal sleep pre-treatment.

This physiologic replacement strategy using a low-dose, slow-release melatonin formulation had no apparent side-effects in the frail elderly. Unlike sedative medications, melatonin treatment did not increase the incidence of falls, confusion or oversedation. It is not known whether an immediate-release formulations at the same dose would have the same effects, positive or negative.

These results indicate that melatonin may be increasing sleep in the responders by increasing sleep duration by a chronobiologic mechanism, *i.e.*, by inducing a corrective phase advance in the patients' endogenous melatonin phase response curve. The two responding patients were sleeping for a significant amount of time during the day, consistent with having a phase delay in their circadian rhythms. Melatonin administered at 2000 hrs would be expected to have a phase-advancing effect on these patients' endogenous melatonin circadian rhythm, which would be expected to correct their phase delay. The results obtained were consistent with these expectations based on the patients' circadian phase response curves.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

**WHAT IS CLAIMED IS:**

1. A dosage form comprising a sustained release melatonin formulation wherein the formulation provides from about 1% to about 10% bioavailable melatonin.
- 5 2. The dosage form of Claim 1 wherein the formulation releases melatonin over a period of from about 3 to 10 hours.
3. The dosage form of Claim 1 wherein the formulation produces plasma melatonin concentrations in an animal that are from about 25 to about 300 pg/ml over a period of at least 3 hours.
- 10 4. The dosage form of Claim 1 wherein the formulation produces a normal nighttime melatonin concentration in an animal.
5. The dosage form of Claim 1 wherein the sustained release melatonin formulation comprises:
  - 15 (a) a sugar-based core comprising less than about 2 mg of melatonin where the melatonin is about 0.05 to 2% by weight of the core; and
  - (b) about 5 to 25% by weight of ethylcellulose coating comprising ethylcellulose and a plasticizer.
6. The dosage form of Claim 5 wherein the plasticizer is an alkyl sebacate or a trialkyl citrate, where each alkyl is the same or different and represents straight or branched chain alkyl groups having 1-6 carbon atoms.
- 20 7. The dosage form of Claim 5 wherein the core additionally contains from about 0.05 to 1% by weight of polyvinylpyrrolidone and 0.01 to 1% by weight of hydroxypropylcellulose.
- 25 8. The dosage form of Claim 5 wherein the core comprises about 0.3% by weight of melatonin.
9. A dosage form according to Claim 1 that is orally administered.
10. The dosage form of Claim 1 wherein the amount of melatonin in the composition is less than about 1 mg.
- 30 11. Use of the dosage form of Claim 1 for preparing a medicament for treating a patient suffering from a melatonin deficiency.

12. A use according to Claim 11 wherein the patient is an elderly patient.

13. The use according to Claim 12 wherein the patient is an Alzheimer's disease patient.

5       14. A use according to Claim 11 wherein the dosage form is administered to the patient from about 4 to about 17 hours before a time characterized as the patient's endogenous melatonin onset time.

10       15. A use according to Claim 11 wherein the dosage form is administered to the patient from about 6 to about 19 hours before a time characterized as the onset time of the patient's normal or desired sleep phase.

Figure 1

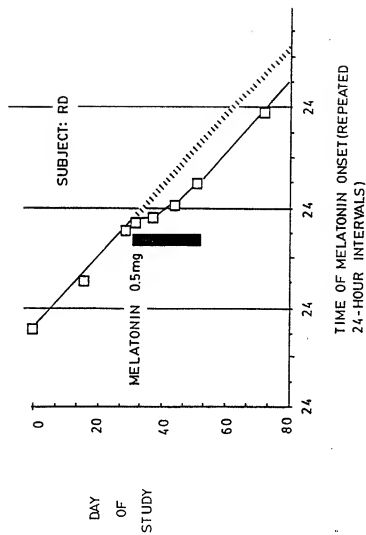
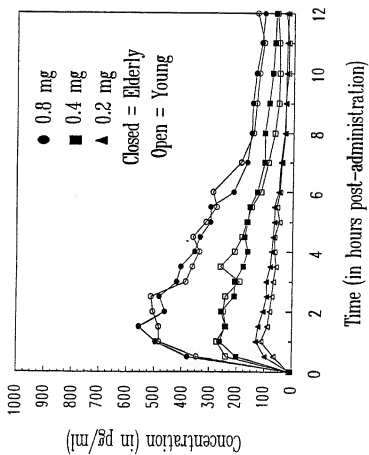


Figure 2



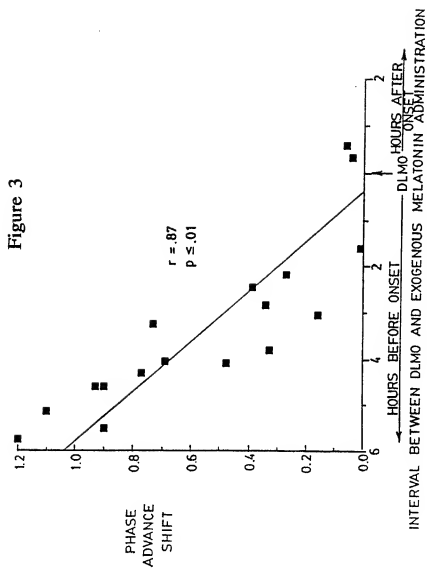


Figure 4

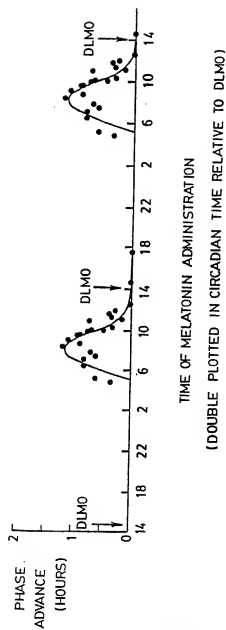


Figure 5

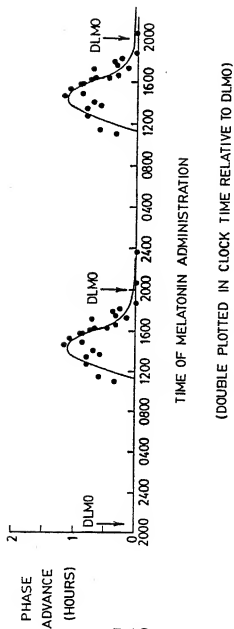
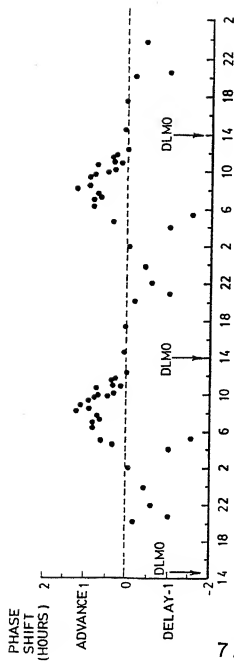






Figure 7



TIME OF MELATONIN ADMINISTRATION  
(MULTIPLE PLOT IN CIRCADIAN TIME RELATIVE TO DLMO)

## INTERNATIONAL SEARCH REPORT

 International Application No  
 PCT/US 94/08365

 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 A61K31/40 A61K9/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 126 630 (MONASH UNIVERSITY) 28 November 1984 cited in the application see claims see page 16, line 28 - line 33 see page 17, line 1 - line 3 ---	1-15
A	EP,A,0 518 468 (NEURIM PHARMACEUTICALS) 16 December 1992 cited in the application see claims 1-11 see page 6, line 3 - line 19 ---	1-15
A	WO,A,85 03227 (GENE LINK) 1 August 1985 see claims 1-13 see examples 1-17 --- -/-	1-15

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A\*" document member of the same patent family

3

Date of the actual completion of the international search

19 December 1994

Date of mailing of the international search report

29. 12. 94

Name and mailing address of the ISA

 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2200 HV Rijswijk  
 Td. (+31-70) 340-2040, Tlx. 31 651 epo nl,  
 Fax (+31-70) 340-3016

Authorized officer

Scarponi, U

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/08365

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 945 103 (M.COHEN) 31 July 1990 see claims 1,3,11 see column 5, line 54 - line 68 see column 6, line 15 - line 25 -----	1-15
A	EP,A,0 246 910 (GENE LINK) 25 November 1987 see claims see examples 1-6 -----	1-15

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/08365

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0126630	28-11-84	CA-A-	1251141	14-03-89
		US-A-	4665086	12-05-87
		AU-A-	2797784	22-11-84
		US-A-	4600723	15-07-86
EP-A-0518468	16-12-92	NONE		
WO-A-8503227	01-08-85	AU-B-	571922	28-04-88
		AU-A-	3811185	01-08-85
		CA-A-	1232800	16-02-88
		EP-A-	0169856	05-02-86
		US-A-	4738679	19-04-88
US-A-4945103	31-07-90	EP-A-	0514373	25-11-92
		GR-A-	90100024	07-06-91
		IL-A-	93053	27-02-94
		WO-A-	9112007	22-08-91
EP-A-0246910	25-11-87	DE-D-	3789332	21-04-94
		ES-T-	2052561	16-07-94
		US-A-	4882137	21-11-89